Thus we see that work upon the roots of this order, especially as to the meristem, has been very limited. In the parts of this paper which follow I have endeavored to point out certain types of structure, as I have found them in the roots of the native species of *Ranunculaceæ*.

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Method for obtaining pure cultures of Pammel's fungus of Texas root rot of cotton.[1]

GEO. F. ATKINSON.

It is not a very difficult matter to obtain artificial pure cultures of spore producing fungi which grow readily in artificial nutrient media. But when we meet with forms of fungi, the spore production of which is unknown, quite a serious difficulty is encountered since spores of other fungi, as well as numerous bacteria, are so apt to be securely lodged in the strands of the mycelium. This serious difficulty is increased when the fungus in question shows a decided aversion to growing on the usual artificial media. The fungus of Texas root rot of cotton, described by Pammel in Bulletin no. 7 of the Texas Agricultural Experiment Station, has not yielded to the methods usually resorted to in obtaining pure cultures.

After trying various methods Pammel failed to obtain a pure culture. In one case threads of the fungus were swept with a camel's hair brush with the hope of obtaining spores. A "pure culture of some fungus" was obtained but its morphological characters were unlike those of the fungus found on the roots of cotton.

During the summer of 1891, at Auburn, Ala., I made several attempts from fresh material received from Texas to induce the fungus to part its hold on the cotton roots and grow under my care and observation. All attempts at that time failed. Affected roots were placed on sand in moist chambers and the fungus strands grew in several cases from four to six inches out over the surface of the sand. When portions of these strands were transferred to nutrient media they failed to grow.

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[1]Paper presented before the Botanical Section of the Am. Assoc. Agr. Coll. and Exp. Sta. New Orleans, Nov. 16, 1892.

In the summer of 1892 I undertook the work again since my labors in the south were soon to end and I was very anxious to obtain a pure culture of this extremely interesting and important organism. I was well supplied with fresh material which was being received once or twice each week.[2] Most of the roots were sent by Mr. R. D. Blackshear, to whom I am especially indebted for the patience and care manifested in gathering and shipping the specimens. Each root was wrapped separately with moist paper retaining a small portion of the earth next it, and several such roots then bound into a single package. They were usually received two days after being removed from the ground. While the roots were en route I prepared several large moist chambers in the following way: A layer of sand about one-half inch deep was placed in the lower vessel and covered with four thicknesses of filter paper. The sand and paper were then well moistened with distilled water, the cover placed in position, being elevated somewhat from the rim of the lower vessel by two tufts of moist cotton to allow free movement of air and steam, this precaution being necessary to avoid breaking the glass while being sterilized. The moist chambers were then piled in a large dry oven, the temperature of which was raised to 140°C. for an hour or two on two successive days. The filter paper was first perforated in several places to prevent its being raised from the sand by steam. When sterilization was complete the tufts of cotton separating bottom and cover were removed.

On receipt of the roots they were carefully unwrapped, the earth removed, the roots rinsed with distilled water, cut in sections about 5^{cm} long and placed horizontally on the filter paper, four or five sections in each moist chamber.

In two or three days the strands of the *Ozonium* could be seen growing out over the filter paper for 4^{cm} to 6^{cm} away from the root. The strands were examined microscopically to determine the fungus. Sterilized glass slides were now placed at the advancing edge of the strand or weft, and upon these were placed small sections of cotton roots, which had been previously boiled and then steamed for several hours for three or four successive days, to thoroughly sterilize them. Sections of such roots about 3^{cm} long were placed, with the aid

[2] Through the kindness of Prof. Geo. W. Curtis, Dir. of the Agri. Exp. Sta., Mr. R. D. Blackshear, Navasota, and Mr. W. H. Farley, Hutto, Texas.

of forceps reddened in the flame, upon the sterilized glass slide, so that one end came in contact with the weft or strand of the *Ozonium*.

At the same time similar sections of sterilized cotton roots were placed in test tubes and partly imbedded in moist sand or partly immersed in distilled water, the tubes with their contents afterwards being thoroughly steam sterilized.

In from twenty-four to forty-eight hours the *Ozonium* strands would bite hold of the bait placed before them and secure such firm hold that the section of root could be transferred bodily to the prepared culture tubes, placing the end containing the growth in contact with the sterilized root already in the tube. Sterilized sweet potatoes were also used in test tubes as a medium upon which to place the transplantings.

Since the fungus grew rather slowly, and there was always danger that it might be contaminated by other fungus threads which had crept along with it, or with bacteria, some thirty or forty moist chambers during a period of six weeks were used, and more than 200 baits were set for the fungus. Out of this number seventy-five baits, which were promising, were transferred to roots in the culture tubes, and from these four or five finally proved to be pure. From these, baits could now be easily handled, so that in the course of two weeks I had multiplied the cultures to the number of fifty.

Great difficulty was encountered in baiting in such a way as to avoid contamination from strands and wefts of *Œdocephalum*, several species of *Fusarium*, *Penicillium*, *Mucor* and some non-fruiting forms, which also grew out from the roots and crept over the filter paper. In several cases bacteria were starved out by making the medium slightly acid with the use of lactic acid.

The *Ozonium* on artificial media, as sterilized cotton roots or sweet potatoes, grows readily after once obtaining a firm hold, and possesses all the characteristics observable in a natural condition upon cotton roots. Being free from obstructions and hindrances which it encounters in nature, the growth is perhaps much more compact, numerous strands uniting to form a broad weft, but the peculiar strands are present, as well as the characteristic branching setæ.

In the first transplantings the fungus grew with difficulty, since I did not have the conditions perfect, but as with expe-

rience I became more and more familiar with its habits, I found it an easy matter to cultivate it with certainty and in profusion.

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A vacation in the Hawaiian Islands.

DOUGLAS HOUGHTON CAMPBELL.

(Concluded from p. 416.)

Of the trees of this lower forest region, much the most conspicuous is the Aleurites Moluccana, a euphorbiaceous tree, called by the natives kukui, with pale silvery green foliage which makes it noticeable at a long distance. The large oily seeds are used as food; and, formerly at least, the expressed oil was used for various purposes. A little higher up, the koa (Acacia koa), one of the commonest forest trees, abounds. This has phyllodia, like so many of the Australian acacias, and it is the principal timber tree, the wood being not unlike mahogany in appearance. Another conspicuous tree of the higher forest region is the ohia, or mountain apple (Eugenia Malaccensis), one of the Myrtaceæ, a medium-sized tree with beautiful crimson fruits not unlike a bell-flower apple in shape. The pulp is white and watery, pleasant to the taste, and very refreshing. Higher still, the related Metrosideros is very abundant, and with its grey-green leaves and scarlet feathery flowers is a striking object.

Owing to the almost constant rains, most of the valleys are traversed by permanent streams, and the floors of these valleys are very productive. Here are found the principal taro plantations. The taro plant (Colocasia antiquorum), familiar enough in American gardens under the name of Caladium esculentum, is the food staple of the great majority of native Hawaiians. Its large farinaceous tuber, after being deprived of its acrid properties by heat, is either directly baked or boiled for eating, or, more commonly, the baked taro is ground up with water into a sort of porridge, allowed to ferment, and served in the form of "poi". This is a sticky, unpleasant-looking mess, which, nevertheless, appears to be very nutritious.